

# Bulletin of the Agricultural Chemical Society of Japan.

## TRANSACTIONS

### A New Bacterium (*Pseudomonas fibrolysis* n. sp.) Decomposing Cellulose.

By Yoshio OTANI.

From the Zymomycological Institute, Imperial College of Agriculture, Tottori, Japan.

(Received Dec. 9, 1938.)

The investigation concerning the bacteria decomposing cellulose has, hitherto, attracted the attention of many workers from various countries, and it is, now, one of the important problems in the bacteriological field. The decomposition of cellulose is generally carried on in nature aerobically or anaerobically, by a large number of different microorganisms. While making some experiments in this field, I isolated a new organism from a piece of decaying cloth, found in a pond. It developed and decomposed cellulose under aerobic conditions at ordinary temperature. I continued work with pure cultures, and I have described the morphology and other properties of this new organism, namely, *Pseudomonas fibrolysis*.

#### 1. MORPHOLOGY.

The organism under observation appeared as small, slender, nonspore-forming rods. Branching or chain formation was not observed. It was Gram negative. In stained preparation from nutrient agar culture, after five days, the mean dimensions of the cells were found to lie in the region of  $1.8\mu$  along the major axis, while the mean diameter is from  $0.5\sim 0.6\mu$ . Endospore was not observed. The organism is actively motile with side polar flagella.

#### 2. CULTURAL CHARACTERISTICS.

These were studied on nutrient agar plates and slants, nutrient gelatin, and potato cylinder, as well as in nutrient broth, milk, litmus milk, and cellulose enrichment solution ( $\text{K}_2\text{HPO}_4$  1 g,  $\text{MgSO}_4\cdot 7\text{H}_2\text{O}$  1 g,  $\text{NaCl}$  1 g,  $\text{CaCO}_3$  2 g,  $\text{KNO}_3$  2 g, a piece of filter paper and water 1,000 cc). All cultures were incubated at  $22^\circ\text{C}$  to  $28^\circ\text{C}$ .

Nutrient agar plate: The colonies formed on this medium had various sizes, from 3 to 5 mm in diameter, after three days incubation. The surface colonies were round or roundish with well defined elevated edges, and faintly shining. Internal colonies were coarsely granular. A yellow colour was presented in some



cultures but not in others.

**Gelatin plate:** The colonies formed on this medium were round or almost round, elevated, opaque, and short-haired along the border. An opaque greasy colour was observed.

**Agar slant:** The surface growth appeared moderately, after five days, and condensed water was cloudy.

**Gelatin stab:** The gelatin was not liquified and stab canal was thread-like.

**Nutrient broth:** There was no surface film and only a slight amount of sediment formed. Slight to moderate turbidity appeared after from two to three days incubation. Odor was absent.

**Milk:** Curd or peptonization not observed.

**Litmus milk:** Growth is slow. After from three to five days incubation, no reddening occurred in the medium. The culture was incubated for twenty days, and litmus was not reduced.

**Potato cylinder:** The culture gave a smooth grayish brown growth along the line of inoculation.

**Cellulose enrichment solution:** After a very few days, namely from two to four days, paper was reduced to a white pulp-like mass made up of very short disintegrated fibers which became distributed through the solution on slight agitation.

### 3. BIOCHEMICAL PROPERTIES.

**Nitrate reduction:** The organism was cultivated in Dunham's solution and in nitrate solution containing 0.2 per cent potassium nitrate. Both gave a positive reaction for nitrite.

**Ammonia production:** Neither of the above mentioned media produced ammonia.

**Gelatinolytic action:** The gelatinolytic action was tested on nutrient gelatin stab cultures. The organism was incubated at 18°C for twentyfive days, and grew in the culture, both on the surface and along the stab line, but the gelatin was not liquified.

**Production of hydrogen sulphide:** A lead acetate paper suspended over the culture media became slightly black after one week or ten days.

**Indol production:** Indol was formed in nutrient broth and peptone water cultures.

**Gas production:** Gas was not produced.

**Oxygen relation:** No growth under anaerobic conditions, and, in all cases, the decomposition of the paper was limited to the part immersed in the medium.

### 4. RELATION TO TEMPERATURE.

About the relations to temperatures we tried the following experiments, viz., the determination of (1) the optimum temperature for cellulose decomposition, and (2) the growth relation of the organism. The culture media used for the determination of optimum temperature for the cellulose decomposition was the enrich-



ment solution, and for the growth relation of the organism was nutrient agar.

The experiments were performed at different temperatures, namely, 0~40°C, as follows:

Temp. (°C)	Cellulose decomposition	Growth	Temp. (°C)	Cellulose decomposition	Growth
0	—	—	28	++	++
4~6	—	—	32	—	++
10	+	+	37	—	—
22	++	++	40	—	—

At the decomposition of cellulose, the most vigorous action took place between 22°C to 28°C. The growth was observed between 10°C to 37°C, the most suitable temperature being 22 to 32°C, and above 40°C and below 6°C no growth was observed.

##### 5. RELATION TO SOURCES OF NITROGEN.

The comparative value of various forms of nitrogen, for the bacterium, was examined at 28°C during ten days, and the result is shown in the following table. The experiment was carried out with 0.2 per cent of following nitrogen forms, together with an enrichment medium and a filter paper.

Nitrogen forms	Cellulose decomposition, (Days)	Nitrogen forms	Cellulose decomposition, (Days)
Ammonium sulphate	4	Potassium nitrite	—
Ammonium nitrate	3	Asparagin	—
Ammonium chloride	3	Peptone	5
Potassium nitrate	4	Liebig beef ex.	7

From the above table we find that according to the forms of nitrogen there have appeared widely different values for the cellulose decomposition. The most suitable sources of nitrogen are ammonium nitrate and ammonium chloride, while potassium nitrite and asparagin give poor results.

##### 6. TAXANOMIC RELATIONSHIP.

This bacterium resembles *Bacillus aquatilis* LUSTIG but the latter cannot grow at a temperature higher than 23 to 25°C and it shows no signs of cellulose decomposition. Then this microörganism is recognized as a new species, namely, *Pseudomonas fibrolysis* n. sp.

**ABSTRACTS**

from

**TRANSACTIONS published in JAPANESE**

(Pages refer to the Japanese originals of this volume unless otherwise noticed)

**On the Method of Quantitative Estimation of Glutathione  
in Tissue by Okuda and Ogawa and its  
Modification by Fujita and Numata.**

(Biochemical Studies on Glutathione. The Eighth Report.)

(pp. 1~7)

By Masayoshi OGAWA.

(Department of Nutrition, College of Medicine, Nippon University, Received Oct. 4, 1938.)

Several years ago (1933) Okuda and the author established a method of estimating reduced and oxidized glutathione in tissue and in biological fluids based on the same principle as that of so called Okuda's Iodine Method for the determination of cystein and cystin (1929). In this method, first of all, protein is removed by means of sulphosalicylic acid and the filtrate is divided into two portions. In one portion reduced glutathione is estimated directly by the titration at 0°C with  $M/10,000$   $KIO_3$  solution, in the presence of certain quantities of iodid and starch. In the other, oxidized glutathione, after being reduced into reduced form by boiling the acid solution with zinc powder, is estimated in the same way. Subsequently (1938) the author corrected the iodate titration value for ascorbic acid and others by means of 2, 6-Dichlorophenol-indophenol treatment.

However (1938) Fujita and Numata reported a method for the determination of glutathione. Their method consists of titrating reduced glutathione in metaphosphoric acid filtrate with  $N/1,000$   $KIO_3$  at 0°C in the presence of KI and starch and of reducing oxidized glutathione by means of  $H_2S$  in the acid solution for 12 hours at room temperature before titration, thus iodate titration value was corrected by means of ascorbic acid oxidase treatment.

Accordingly their method is entirely based upon the same principle as that of the above mentioned Okuda and the author's method determining GSH and GS·SG compound. The chief differences between their method and our's are as follows:— 1) According to their method the extraction is made with metaphosphoric acid. 2) The reduction is carried out by means of  $H_2S$  for 12 hours. 3) The results of iodate titration value are corrected with ascorbic acid oxidase treatment.

In the present communication, in order to ascertain the accuracy of their method the author made some experiments with the following results:—



Table I. Reduction of GS·SG into GSH by means of Zn powder and H<sub>2</sub>S.

Glutathione found, (mg %)		Reduction by means of			
		Boiling with Zn dust for 20 minutes.		Saturating with H <sub>2</sub> S for 12 hours.	
Sample	GSII	GS·SG	Total	GS·SG	Total
Liver	178	13	191	7	185
Heart	44	82	126	80	124
Lung	144	33	177	31	75
Kidney	130	50	180	46	176
Spleen	127	145	272	125	252

Table II. GSH value of yeast, which was corrected by means of 2, 6-Dichlorophenol-indophenol or ascorbic acid oxidase treatment.

GSH found (mg %)	GSH values: correction was made by means of	
	2, 6-Dichlorophenol-indophenol.	Ascorbic acid oxidase treatment.
I.	650	787
II.	680	735
III.	673	779
Means	668	767

From these above results the author concluded that reduction of GS·SG into GSH by means of H<sub>2</sub>S treatment is nearly as effective as that of Zn dust, but GS·SG value of the former is slightly lower compared with that of the latter, and the reduction with H<sub>2</sub>S treatment requires a longer time, while by means of Zn powder it is rapid and more advantageous.

Iodate titration value (GSH) of tissue extract which was corrected by means of ascorbic acid oxidase treatment gives an extraordinary higher result compared with that of 2, 6-Dichlorophenol-indophenol in the case of dry beer yeast. Therefore, by means of ascorbic acid oxidase treatment the correction for iodate titration value of general tissue and biological fluids, with some exceptions, seems to be incomplete which results in inaccurate estimation.

Okuda, Ogawa: The Journal of Biochemistry, Vol. XVIII, No. 1, 75, 1933.

Fujita, Numata: Tokyoiishinshi, No. 3093, 1938.

#### Statistic Studies of Soils. (IV)

(pp. 8~10)

By Dr. MISU-Hideo.

(Agricultural Experiment Station, Government General of Tyosen, Received June 24, 1938.)

## On the Yeasts of the Genus *Schizosaccharomyces* indigenous to Iô-Island. (Ogasawara Islands)

(pp. 11~18)

Kin-ichirô SAKAGUCHI and Yosihisa ÔTANI.

(Tokyo Imperial University, Received Nov. 15, 1938.)

The following 4 new varieties of *Schizosaccharomyces pombe* were isolated from the fermented molasses from Iô-island, one of the main island of the Ogasawara islands.

*Schizosaccharomyces pombe* var. *ogasawaraensis* I. nov. var.

*Schizosacch. pombe* var. *ogasawaraensis* II. nov. var.

*Salizosacch. pombe* var. *iotoensis* I. nov. var.

*Schizosacch. pombe* var. *iotoensis* II. nov. var.

Special studies were made on their particular enzymic activities, i. e. the ureolysis, the liquefaction of gelatin and the fermentation of dextrins and inulin.

## Studies on the Enzymes of Lactic Acid Bacteria. (Part II).

On Lactic Dehydrogenase of Lactic Acid Bacteria.

(pp. 19~24)

By Kakuo KITAHARA.

(Department of Agriculture, Kyoto Imperial University, Received Nov. 10, 1938.)

It was found that *dl*-lactic acid was always dehydrogenated by all kinds of lactic acid bacteria under investigation in which resting cells of the bacteria were employed in the presence of methylene blue. However, bacterial cells were found to show complete optical specificities in oxidizing optically active lactic acids, as illustrated in the following table.

Species of Bacteria	Dehydrogenation of		
	<i>d</i> -Acid	<i>l</i> -Acid	<i>dl</i> -Acid
<i>L. sake</i> ( <i>d</i> -former)	+	—	+
<i>Leuconostoc</i> ( <i>l</i> -former)	—	+	+
<i>L. plantarum</i> ( <i>dl</i> -former)	+	+	+

The author proposed, therefore, that the so-called lactic dehydrogenase could be divided into *d*- and *l*-enzymes, and concluded that *L. sake* would contain only *d*-enzyme and *Leuc. mesenteroides* would, on the contrary, contain only *l*-enzyme.

When the bacterial cells were treated with acetone, *L. plantarum* was proved to contain only *l*-lactic dehydrogenase, so that the reason why the bacteria attacked both *d*- and *l*-acids was attributable to the presence of racemiasse which had been inactivated by acetone.



For the dehydrogenated product of lactic acid, pyruvic acid was easily isolated in a yield of about 60%. But neither acetaldehyde nor acetic acid were obtained. This proves that carboxylase never existed in the lactic acid bacteria, whenever they revealed homo- or hetero-fermentations.

## **Untersuchungen über die Herstellungsmethode des Bagasse-Zellstoffes. (II)**

Über dem Kochen der Bagassen mit Wasser unter Druck und  
den Bagasse-Zellstoff nach dem Natronverfahren.

(ss. 25~32)

Von Syosuke TANAKA und Munco KOORIYAMA.

(Department of Industry, Government Research Institute, Taiwan, Japan, Received Oct. 31, 1938.)

## **On the Alcohol-Manufacture from Jerusalem Artichoke. (Part VIII)**

Influences of Storage on the Alcoholic Fermentation  
of Jerusalem Artichoke.

(pp. 33~42)

By Toshinobu ASAI and Tetsuo YUKINOURA.

(Agricultural Chemical Laboratory, Morioka Agricultural College, Japan, Received Nov. 14, 1938.)

## **On the Koji-Amylase.**

Part XII.—Studies on Some Protective Substances for the Heat  
Inactivation of  $\alpha$ - and  $\beta$ -Amylases or Maltase, and on the  
Regeneration of these Enzymes being Inactivated by Heat.

(pp. 43~49)

By Yuzo TORUOKA.

(Tsunekichi Okura Brewery, Received Nov. 15, 1938.)

It was ascertained that Ca-ion revealed very remarkable protective action upon the heat inactivation of  $\alpha$ -amylases, since in the presence of  $\text{CaCl}_2$ , the activity of  $\alpha$ -amylase solutions of pH=5.5~8.0 (especially at pH 7.0~7.5) was always found to be perfectly protected from heating at 40° for 20 hours, while 40~100 % of its activity was lost by the same treatment without the addition of  $\text{CaCl}_2$ . This protective action was again revealed by the other Ca-salt as  $\text{CaHPO}_4$ , and very remarkable protection was found even in such a lower concentration of Ca-ion as  $M/500$ .

Any noticeable protective action was not observed with  $\text{NH}_4$ -, Na-, K- and

Mg-ions. Although Ca-ion produced very much the same result with the preparations of  $\alpha$ -mylases obtained from various materials including pancreatin, barley malt, Takadiastase and Saké-Koji, no evidence for the protective action of Ca-ion was obtained with maltase of Saké-Koji or with  $\beta$ -amylase prepared from wheat and Saké-Koji.

Starch, protein and their decomposition products revealed protective action upon  $\alpha$ -amylase in the order of  $\text{CaCl}_2 > \text{starch} > \text{dextrin} > \text{peptone}$ , egg-white  $>$  maltose  $>$  glucose, and very low degree of protection of maltase and of  $\beta$ -amylase was produced by these organic substances.

A part (nearly 5%) of  $\alpha$ -amylase was regenerated within 2 hours by cooling to  $25\sim 27^\circ$  from its inactive state produced by heating at  $80^\circ$  for 15 minutes.

More remarkable (nearly 70%) regeneration was obtained with maltase and  $\beta$ -amylase when these enzymes were inactivated by heating at  $90^\circ$  for 30 minutes.

Noticeable inhibition on the regeneration of inactive maltase and  $\beta$ -amylase was observed with the organic substances mentioned above.

## Studien über Sericin.

### III. und IV. Mitteilung.

(ss. 50~55)

Von Takeo ITO und Kozo KOMORI.

(Aus dem chemischen Laboratorium der Seidenbau-Hochschule, Kyoto, Eingegangen am 8, 11, 1938.)

III. Nachdem der N-Gehalt von Sericin-A und Sericin-B festgestellt worden ist (Diese Zeitschr. 13, 115, 1937), haben wir durch "van Slyke's" Analysierung versucht derselben N-Verteilung zu bestimmen. Die erhaltenen Resultate sind in Tab. 1 zusammengestellt.

Tabelle 1. N-Verteilung von Sericin-A und Sericin-B.

	Ammoniak-N	Melanin-N	Zystin-N	Arginin-N	Lysin-N	Histidin-N	Mono-amino-N	Nicht-amino-N	Summe
Sericin-A	12.14	0.17	0	9.15	4.70	2.71	70.10	0.61	99.53
Sericin-B	10.22	0.90	0	9.68	4.72	3.80	69.19	1.14	95.65

Es ist leicht ersichtlich, dass sich die beiden Sericinfractionen dadurch am merklichsten voneinander unterscheiden, dass der Melanin-N-Gehalt vom Sericin-B, im Vergleich zu demjenigen vom Sericin-A, weit grösser ist.

Ferner wurden Untersuchungen bezüglich Gehalts an Kohlenhydrat und Amino-Kohlenhydrat der genannten Proteine durchgeführt.

Dabei wurde das Kohlenhydrat nach Tillmans und Philippi (Biochem. Z. 215, 36, 1929), und das Aminokohlenhydrat nach Nilsson (Biochem. Z. 285, 386, 1936) colorimetrisch bestimmt. Es ergab sich, wie aus Tab. 2 ersichtlich ist, dass die



beiden Kohlenhydratsorten in höherem Masse im schwerlöslichen Sericin-B, als im leichter löslichen Sericin-A, nachweisbar sind. Diese Tatsache wird mit den oben berichteten Resultaten über Melanin-N-Gehalt in engem Zusammenhang stehen.

Tabelle 2. Kohlenhydrat- und Aminokohlenhydrat-Gehalt von Sericin-A und Sericin-B.

	Kohlenhydrat als Glukose berechnet.	Aminokohlenhydrat als Glukosamin berechnet.
Sericin-A	0,45 %	0,37 %
Sericin-B	1,08 %	1,25 %

IV. Wir haben früher mitgeteilt, dass durch Behandeln der Kokonschicht mit heissem Wasser, Ammoniak entsteht. Um die Frage, ob dieses aus dem Sericin der Kokonschicht oder aus deren Fibroin entsteht, aufklären zu können, wurde die vorliegende Untersuchung angestellt.

0,2 g Sericin wurden mit 100 ccm Wasser in einem zweckmässig konstruierten Apparat erhitzt, und dabei wurde das entstehende Ammoniak bestimmt. Folgende Tabelle gibt die Ergebnisse der Versuche wieder.

Tabelle 1. Ammoniak Abspaltung: mg Ammoniak-N pro 100 g Sericin.

Temperatur	100°		110°		120°		130°	
	Sericin		Sericin		Sericin		Sericin	
Erhitzungsdauer	A	B	A	B	A	B	A	B
1 Std.	—	—	150	98	—	—	—	—
2	122	69	231	155	394	304	666	505
4	220	141	414	262	662	444	1029	791
8			586	404				

Man sieht, dass sowohl Sericin-A als auch Sericin-B, wenn sie im Wasser erhitzt werden, Ammonia abgeben, und zwar das Erstere in höheren Masse als das Letstere.

Versuche mit solchem Fibroin, welches aus Kokonschicht durch vorsichtige Degummierung hergestellt wurden, folgendermassen ausgeführt: 2 g Fibroin wurden mit 100 ccm Wasser 2 Stunden lang bei einer gegebenen Temperatur erhitzt, dann nach dem Abkühlen filtriert und gut gewaschen. Filtrat und Waschwasser wurden vereinigt und analysiert. Mit dem zurückgebliebenen Fibroin wurde die gleiche Operation wiederholt. Tab. 2 gibt einen Teil der erhaltenen Resultate wieder.

Tabelle 2. Der bei der Heisswasser-Behandlung des Fibroins in Lösung gehende Gesamte- und gleichzeitig entstehende Ammoniak-Stickstoff in mg pro 100 g Fibroin. Die Erhitzung dauerte jedesmal 2 Stunden.

Temperatur	100°	110°	120°		130°
	Ammoniak-N	Ammoniak-N	Gesamte-N	Ammoniak-N	Ammoniak-N
Bei der 1. sten Behandlung	14	30	512	51	82
" " 2 " "		22	383	31	
" " 3 " "		13	383	23	
" " 4 " "		18	362	23	
" " 5 " "		13	353	22	
Summe		96	1993	150	



Tab. 2 lehrt, dass das Fibroin bei selbst wiederholtem Erhitzen im Wasser wenn auch nur in geringer Menge Ammoniak abgibt. Bei Berücksichtigung der Tatsache, dass die Kokonschicht hauptsächlich aus 20~25% Sericin und 75~80% Fibroin besteht, kann man aus den oben erwähnten Ergebnissen folgern, dass das bei der Heisswasser-Behandlung der Kokonschicht entstehende Ammoniak, grösstenteils aus Sericin, aber auch teilweise aus Fibroin stammt.

Es wurde ferner festgestellt, dass das Fibroin Kohlenhydrat enthält, und zwar 2 mg (als Glukose berechnet) pro 100 mg Fibroin-N. Es konnte nachgewiesen werden, dass dieser Kohlenhydratgehalt dann abzunehmen beginnt, wenn das Fibroin in der angegebenen Weise wiederholt mit heissem Wasser behandelt worden ist. Andererseits erkennt man aus Tab. 2, dass im Teil von etwa 10% des ursprünglich im Fibroin enthaltenen Stickstoffs nach 5 maligem Wiederholen der genannten Behandlung bei 120°, in Lösung gegangen ist. Das beweist, dass somit ein Teil des Fibroins samt dem Kohlenhydrat aufgelöst wird.

Ohara (Sc. Pap. I. P. C. R. Japan, 21, 104, 1933) hat durch eingehende Untersuchungen sehr wahrscheinlich gemacht, dass die beim Erhitzen im Wasser auftretende Beschädigung des Fibroins nicht auf die Veränderung dessen Krystallstruktur, sondern auf die des nichtkrystallinen Teils zurückzuführen sei. Damit dürfen wir annehmen dass die oben erwähnten Verhältnisse mit Ohara's Feststellungen in engem Zusammenhang stehen. Wenn also der amorphe Teil des Fibroins beim Erhitzen im Wasser teilweise in Lösung gehen würde, während die krystallinische Mizelle ungelöst zurückbliebe, so würde der N-Gehalt des Fibroins im Verlaufe der genannten Behandlung geringermassen zunehmen, denn die krystallinische Mizelle des Fibroins soll nach Mark und Meyer u. a. nur aus kleinemolekularen Aminosäuren wie Glykokoll und Alanin bestehen. Tatsächlich wurde gefunden, dass dieser sich nach 5 maligem Wiederholen der Erhitzung des Fibroins im Wasser bei 120° von den anfänglichen 18,78% auf 18,95% steigert. Die Zunahme ist zwar geringmässig, dennoch analytisch unverkennbar.

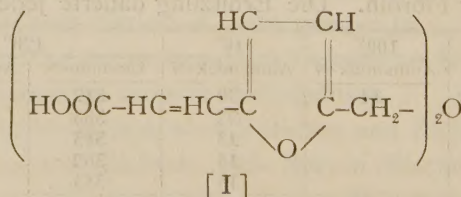
### Synthesis of bis-[5-oxymethylfurfuracrylic acid]-ether.

(pp. 56)

By Kiyosi Aso.

(Tokyo Imperial University, Received Dec. 16, 1938.)

The author synthesised bis-[5-oxymethylfurfuracrylic acid]-ether [I] from  $\omega$ -oxy-methylfurfural-ether by means of Perkin's reaction. It crystallises in brilliant white plates which decompose at 203—204°.





## Decomposition Products of Substance containing Uronic Acid in Autoclave (II).

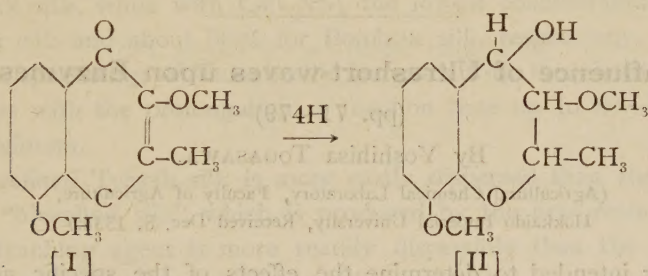
Reduction of Dimethyl-alginetin

(pp. 57~58)

By Kiyosi Aso.

(Tokyo Imperial University, Received Dec. 16, 1938.)

The author obtained tetrahydro-dimethyl-alginetin (2-methyl-3, 8-dimethoxy-4-hydroxy-chroman) [II] by the catalytic reduction of dimethyl-alginetin (2-methyl-3, 8-dimethoxy-chromon) [I]. It forms silky needles, melts at 132°. The acetyl-derivative crystallises in colorless long prisms, melts at 117°.



## Synthesis of 3, 8-dimethoxy-flavone.

(pp. 59~60)

By Kiyosi Aso.

(Tokyo Imperial University, Received Dec. 16, 1938.)

The author synthesised 3, 8-dimethoxy-flavone by heating 2-hydroxy-3,  $\omega$ -dimethoxy-acetophenone (m.p. 66°) with benzoic anhydride and anhydrous sodium benzoate at 160~163° for 3 hrs.. It crystallises in yellow prisms, melts at 156~157°. Its two maximum of absorption spectrum are at frequency ca 3,320 and 3,920.

## Chemical Studies on the Kikyô-root (*Platycodon grandiflorum*, A. Dc.) (Report IV)

On the Haemolytic Action and Toxic Properties of the Kikyô-root

(pp. 61~70)

By Magosaburo TSUJIMOTO.

(Kagoshima Imperial College of Agriculture & Forestry, Received Dec. 8, 1938.)

The object of my experiments was to determine the medicinal value of the kikyô-roots which differ in region, age, variety and preparation. For this purpose

the haemolytic actions and poisonous properties of these samples were determined.

#### SUMMARY

- 1) Wild plants have more active haemolytic power than the cultivated ones.
- 2) Unrinded roots have more active haemolytic power than the rinded ones.
- 3) The intensity of haemolytic power increases according to the age of the plant. Thus: the second- > the first- > the third-year plant.
- 4) Violet-flowering plants have more active haemolytic power than the white-flowering ones.
- 5) The poisonous properties<sup>\*)</sup> of the kikyô-root run parallel with its haemolytic power.

### Influence of Ultrashort-waves upon Enzymes.

(pp. 71~79)

By Yoshihisa TOGASAWA.

(Agricultural Chemical Laboratory, Faculty of Agriculture,  
Hokkaido Imperial University, Received Dec. 8, 1938.)

The author intended to determine the effects of the specific action of ultrashort-waves upon enzymes, avoiding the heat effect caused by electric current.

Two, 5 and 10 m of ultrashort-waves (1,000~1,500 V) was not effective under various conditions on a number of commercial preparations (diastase, papayotin and pancreaslipase). The circuit used in this experiment was the Hertley circuit.

### Biochemical Investigation of Mosaic Disease of Tobacco Plants. V.

On the Ascorbic Acid Content in the Leaves  
of Healthy and Mosaic Plants.

(pp. 80~86)

By Y. OKUDA and K. KATAI.

(Department of Agriculture of Kyushu Imperial University, Fukuoka; Received Dec. 12, 1938.)

### On the Chemical Regeneration of Waste Tussah Silk. (Part I.)

Dispersion of Tussah Silk in Concentrated  
Solutions of Neutral Salts.

(pp. 87~92)

By Chiyoka MIYAHARA.

(Central Laboratory, South Manchuria Railway Co., Dairen, Received, Nov. 28, 1938.)

\*) The experiments were made on fishes.



Tussah silk is the silk secreted by the wild silk-worm, *Antheraea pernyi*, which is widely raised in Manchuria. The yield of waste silk produced by the present reeling process amounts to as much as 40% of the total silk layer of its cocoon. Although many studies have been made on the regeneration of waste Bombyx silk, very few investigations have been reported on those of Tussah silk.

The author has made fundamental studies on the dispersion of waste Tussah silk in concentrated solutions of  $\text{ZnCl}_2$  and  $\text{Ca}(\text{CNS})_2$  with the object of its regeneration in mind. The results obtained are as follows:—

1. The dispersion media have the lower limits of concentration under which both Tussah and Bombyx silks are dispersible. With  $\text{ZnCl}_2$  solution the lower limits of concentration are shown to be about 65% for Tussah silk and about 50 % for Bombyx silk, while with  $\text{Ca}(\text{CNS})_2$  the lowest concentrations are about 50 % for Tussah silk and about 30% for Bombyx silk, respectively.

2. The dispersibility of Tussah silk increases with the elevation of temperature as well as with the prolongation of reaction time up to 50~60 hrs, when it reaches a maximum.

3. The refined Tussah silk is more easily dispersed than the raw silk, and the so-called "bleached silk" which is produced by the new reeling process using  $\text{Na}_2\text{O}_2$  as a bleaching agent is more readily dispersible than the so-called "gray silk" which is made by the old plate reeling method.

## On the Chemical Regeneration of Waste Tussah Silk. (Part II.)

Viscosity of Tussah Silk Solutions

(pp. 93~98)

By Chiyoka MIYAHARA.

(Central Laboratory, South Manchuria Railway Co., Dairen., Received Nov. 28, 1938.)

The author has made studies on the viscosity of Tussah silk solutions when a concentrated solution of  $\text{Ca}(\text{CNS})_2$  was used as a dispersion medium with the following results:

1. The viscosity is greatly influenced by higher concentrations (13~15%). The rate of increase of viscosity is much more remarkable than that at lower concentrations. The viscosity-concentration constant of dispersed Tussah silk is much smaller than that of Bombyx silk.

2. The viscosity decreases according to the elevation of dispersion temperature.

3. The viscosity decreases for a few hours after the dispersion, but increases gradually afterwards. After 1~2 days it reaches a maximum, and then decreases slowly again.

4. Various substances such as phenol,  $\alpha$ -naphthol, urea, glucose, sucrose,

glycerine, glycine and *l*-leucine added to the dispersion system somewhat increase its viscosity.

### The Change in the Starch and Sugar Contents of the Tobacco Leaves after Topping.

(pp. 99~103)

By T. NITO and M. OTA.

(Government Monopoly Bureau, Japan, Received Oct. 28, 1938.)

### Statistic Studies of Soils. (V)

(pp. 104~106)

By Dr. MISU-Hideo.

(Agricultural Experiment Station, Government General of Tyosen, Received June 24, 1938.)

### Partial Oxidation of Starch by Bromine.

(pp. ~ )

By Yoshijiro KIHARA.

(Tokyo Imperial University, Received Dec. 17, 1938.)

Starch paste was oxidized by dropping Br in it in the presence of  $\text{CaCO}_3$  at room temperature. The excess of Br was removed from the reaction product by vacuum distillation, the product was filtered and a small amount of alcohol was added to the filtrate, yielding white precipitate. It showed uronic acid reaction by naphthoresorcin, and was named "Urondextrin". It was soluble in water, gave an acidic reaction and its rotation in aqueous solution was  $[\alpha]_D^{15} + 181.72^\circ$ . It was precipitated by  $\text{Ca}(\text{OH})_2$  or  $\text{Ba}(\text{OH})_2$ , but not by  $\text{CuSO}_4$  nor Fehling's solution. Acetyl derivative, m.p.  $145^\circ$ , was prepared, which also showed uronic acid reaction.

Urondextrin was scarcely hydrolyzed by Takadiastase.